PRODUCTION OF NATURAL PROTEIN USING CHICKEN FEATHER.

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ABSTRACT

A research was conducted on producing protein from chicken feathers. Protein is an important nutrient needed by our body to maintain body structures and important ingredient for cosmetic products. Chicken feathers have elevated keratin protein content and can be a suitable protein source. The main processes are dissolving chicken feathers and separation of proteins. Reducing agents Potassium cyanide, thioglycolic acid and sodium sulfide used for the dissolving process. Ammonium sulfate is used for the separation process. Once the feathers are dissolved ammonium sulfate solution is added to the solution which will precipitate protein. The precipitated protein is washed with water and dissolved in sodium hydroxide solution to obtain protein solution. Sodium sulfide has the highest efficiency in dissolving chicken feathers since the feathers are dissolved in a very short period of time. After the methods of precipitation, washing and dissolving the protein solution obtained is confirmed as pure protein solution by biuret test. An analysis by the Ftir confirmed the presence of carboxyl acid and amino groups only. Thus the sample obtained is true protein since the presence of functional groups is proven. From this research can be concluded that protein can be produced from chicken feathers. Hopefully chicken feathers will be used as a source of protein production in a bigger scale in the future.

ABSTRAK

Penyelidikan dilakukan untuk menghasilkan protein dari bulu ayam. Protein adalah nutrisi penting yang diperlukan oleh tubuh kita untuk menjaga struktur tubuh dan bahan penting untuk produk kosmetik. Bulu ayam mempunyai kandungan keratin protein tinggi dan boleh menjadi sumber protein yang sesuai. Proses utama adalah melarutkan bulu ayam dan pemisahan protein. Kalium sianida, asid thioglycolic dan sodium sulfida digunakan untuk proses larut. Amonium sulfat digunakan untuk proses pemisahan. Setelah bulu dilarutkan larutan amonium sulfat ditambah ke dalam larutan dimana protein berhasil. Endapkan protein dicuci dengan air dan dilarut dalam larutan natrium hidroksida untuk mendapatkan larutan protein. Natrium sulfida mempunyai kecekapan yang terbaik dalam melarutkan bulu ayam sebab bulu dilarutkan dalam masa yang sangat singkat. Selepas kaedah presipitasi, mencuci dan melarutkan protein yang diperolehi dikukuhkan sebagai larutan protein dengan ujian biuret. Sebuah analisa oleh FTIR mengesahkan ada kumpulan karboksil dan kumpulan amino saja di dalam sampel. Jadi sampel yang diperolehi adalah larutan protein benar kerana terdapat kedua dua kumpulan tersebut. Kesimpulanya protein boleh dihasilkan dari bulu ayam. Semoga bulu ayam akan digunakan sebagai sumber pengeluaran protein dalam skala yang lebih besar di masa mendatang.

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LIST OF ABREVIATIONS

UV-Vis	-	Ultra Violet Visual Spectrometer
FTIR	-	Fourier Transform Infrared Spectrometer
IR	-	Infrared

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
%	-	Percent
g	-	Gram
L	-	Liter
rpm	-	Rotation Per Minutes
M	-	Molarity
ml	-	Milliliter
A	-	Absorbance
I	-	Length
c	-	Concentration
ε	-	Molar Absorptivity
cm^{-1}	-	Reciprocal Centimeter
mg	-	Milligram

CHAPTER 1

INTRODUCTION

1.1 Backround

This research is about extracting natural proteins from chicken feathers by using reducing agents that will decrease the stability of keratin fibers in the solid form found in feathers. These reagents will break down disulphide bonds, hydrogen bonds and salt linkages of the keratin fibers in order to dissolve it into natural protein. Currently there is an increasing interest in the development of materials that are environment friendly, obtained from renewable resources. The main renewable materials are obtained from polysaccharides, lipid and proteins.

Proteins are polymers formed by various amino acids capable of promoting intra- and inter-molecular bonds, allowing the resultant materials to have a large variation in their functional properties. Proteins also known as polypeptides are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Protein deficiency is a serious cause of ill health and death in developing countries. Protein deficiency plays a part in the disease kwashiorkor. War, famine, overpopulation and other factors can increase rates of malnutrition and protein deficiency. Protein deficiency can lead to reduced intelligence or mental retardation, see deficiency in proteins, fats, carbohydrates. The protein shortage for food and feed oblige us to look for new protein sources, including waste products.

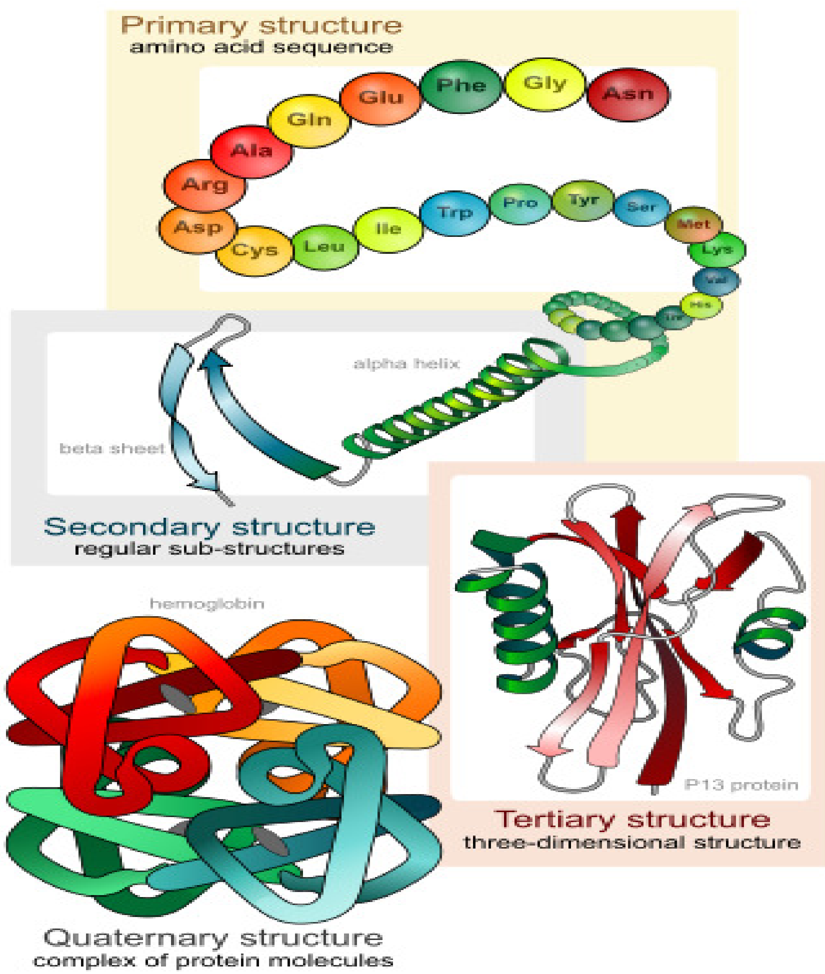

Figure 1.1: The different structures of protein.

Feathers are bio-resource with high protein content (more than 750 g kg-1 crude protein). Poultry slaughterhouses produce large amounts of feathers. Further, burning in special installations is economically ineffective. Uncontrolled disposal of feathers is environmentally unacceptable. The solution of the problem is obligatory since poultry production plays a vital role in the protein supply and also in the agricultural economy for many countries in the world. Five percent of the body weight of poultry is feathers; from a slaughterhouse with a capacity of 50 000 birds daily are produced 2-3 tones dry feathers. The β-keratins in feathers, beaks and claws — and the claws, scales and shells of reptiles — are composed of protein strands hydrogen-bonded into β-pleated sheets, which are then further twisted and cross linked by disulfide bridges into structures even tougher than the α-keratins of mammalian hair, horns and hoof.

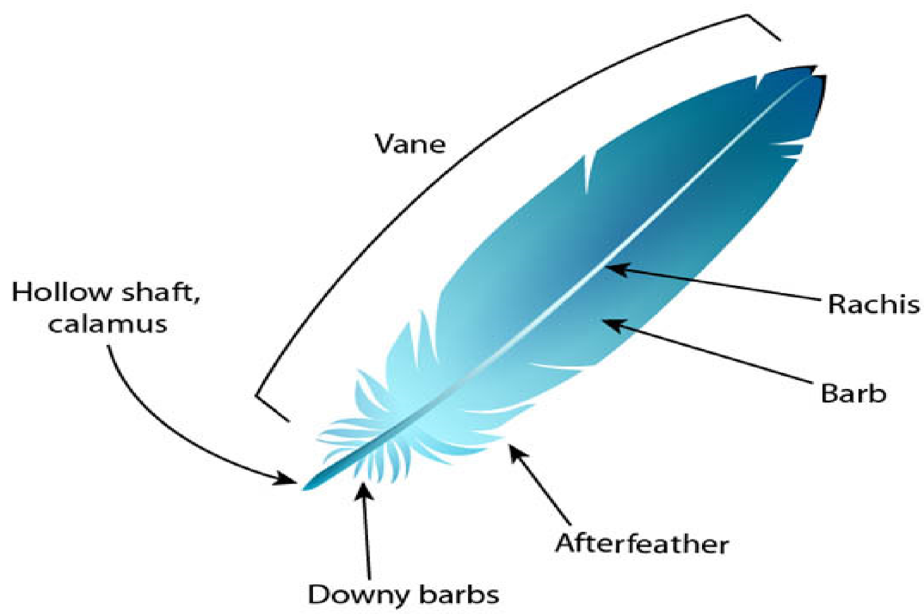

Figure 1.2: Important parts of a feather.

Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan. Over 90% of the dry weight of hair are proteins called keratins, which have a high disulfide content, from the amino acid cysteine. The robustness conferred in part by disulfide linkages is illustrated by the recovery of virtually intact hair from ancient Egyptian tombs. Feathers have similar keratins and are extremely resistant to protein digestive enzymes. Different parts of the hair and feather have different cysteine levels, leading to harder or softer material. Manipulating disulfide bonds in hair is the basis for the permanent wave in hairstyling. The high disulfide content of feathers dictates the high sulfur content of bird eggs. The high disulfide content of hair and feathers contributes to the disagreeable odor that results when they are burned.In chemistry, a disulfide bond is a covalent bond, usually derived by the coupling of two thiol groups. The linkage is also called an SS-bond or disulfide bridge. The overall connectivity is therefore R-S-S-R. The terminology is widely used in biochemistry. Formally the connection is called a persulfide, in analogy to its congener, peroxide (R-O-O-R), but this terminology is obscure. Disulfide bonds are usually formed from the oxidation of sulfhydryl (-SH) groups, especially in biological contexts.

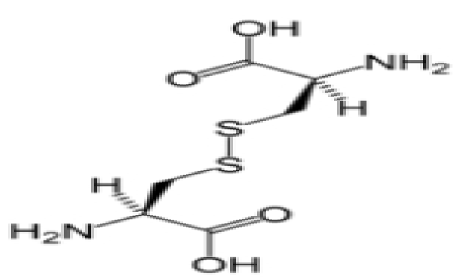

Figure 1.3: Disulfide bond between amino acids Cysteine.

Reductants used in this research for reduction of disulfide groups of feather keratins are thioglycolic acid, potassium cyanide, and sodium sulfide. The reductants act very quickly and without bringing about any other appreciable chemical alteration or damage to the protein yield. Products prepared from the solutions behave as true proteins, and not as products of hydrolysis. Their solutions are precipitated by ordinary protein precipitants such as sulfosalicylic acid, ammonium sulfate and lose this property when digested by trypsin or pepsin.

Thiglycolic acid is the organic compound $HSCH_2CO_2H$. It contains both a thiol (mercaptan) and a carboxylic acid. It is a clear liquid with a strong unpleasant odor. It is simply reduces the disulfide to sulfhydryl groups with no other appreciable chemical change.

Potassium cyanide is an inorganic compound with the formula KCN. This colorless crystalline compound, similar in appearance to sugar, is highly soluble in water. Most KCN is used in gold mining, organic synthesis, and electroplating. Smaller applications include jewelry for chemical gilding and buffing. KCN is highly toxic. The moist solid emits small amounts of hydrogen cyanide due to hydrolysis, which smells like bitter almonds. Not everyone, however, can smell this odor: the ability to do so is a genetic trait. Potassium cyanide reduces feather combined with 0.1N sodium hydroxide.

Sodium sulfide is the name used to refer to the chemical compound Na_2S but more commonly its hydrate $Na_2S{\cdot}9H_2O$. Both are colorless water-soluble salts that give strongly alkaline solutions. When exposed to moist air, Na_2S and its hydrates emit hydrogen sulfide, which smells much like rotten eggs or flatus. The dissolving action of sodium sulfide has been known for a long time and is used industrially.

Ammonium sulfate,$(NH_4)_2SO_4$, is an inorganic salt with a number of commercial uses. The most common use is as a soil fertilizer. It contains 21% nitrogen as ammonium cations, and 24% sulfur as sulfate anions. In fertilizer the purpose of the sulfate is to reduce the soil pH. It is used to purify proteins by altering their solubility. It is a specific case of a more general technique known as salting out.

Copper(II) sulfate is the chemical compound with the formula $CuSO_4$. This salt exists as a series of compounds that differ in their degree of hydration. The anhydrous form is a pale green or gray-white powder, whereas the pentahydrate ($CuSO_4 \cdot 5H_2O$), the most commonly encountered salt, is bright blue. The anhydrous form occurs as a rare mineral known as chalcocyanite. The hydrated copper sulfate occurs in nature as chalcanthite (pentahydrate), and two more rare ones: bonattite (trihydrate) and boothite (heptahydrate). Archaic names for copper(II) sulfate are "blue vitriol" and "bluestone".

Potassium hydroxide is an inorganic compound with the formula KOH. Its common name is caustic potash. Along with sodium hydroxide (NaOH), this colourless solid is a prototypical strong base. It has many industrial and niche applications. Most applications exploit its reactivity toward acids and its corrosive nature. In 2005, an estimated 700,000 to 800,000 tons were produced. Approximately 100 times more NaOH than KOH is produced annually. KOH is noteworthy as the precursor to most soft and liquid soaps as well as numerous potassium-containing chemicals.

Sodium hydroxide (NaOH), also known as lye and caustic soda, is a caustic metallic base. It is used in many industries, mostly as a strong chemical base in the manufacture of pulp and paper, textiles, drinking water, soaps and detergents and as a drain cleaner. Worldwide production in 2004 was approximately 60 million tonnes, while demand was 51 million tonnes. Pure sodium hydroxide is a white solid available in pellets, flakes, granules, and as a 50% saturated solution. It is hygroscopic and readily absorbs water from the air, so it should be stored in an airtight container. It is very soluble in water with liberation of heat. It also dissolves in ethanol and methanol, though it exhibits lower solubility in these solvents than does potassium hydroxide. Molten sodium hydroxide is also a strong base, but the high temperature required limits applications. It is insoluble in ether and other non-polar solvents. A sodium hydroxide solution will leave a yellow stain on fabric and paper.

1.2 Problem Statement

- Determine the best reducing agent that would produce higher amount of protein.
- Establish an extraction method that would have a minimal damage on chicken feather's protein.
- Use suitable protein precipitant for higher protein purification.
- The substance obtained at the end of experiment act as true protein.
- Analysis on the protein obtained.

1.3 Objectives

- Produce natural protein from chicken feather as an alternative source of natural protein.
- Find the keratin reducing (dissolution of chicken feather) efficiency of each reductants by comparing the amount of protein obtained.
- Find a suitable method to purify protein obtained.

1.4 Scope of Project

- Study the solubility of chicken feather keratin under different reductants and obtain natural protein by reducing the keratin in chicken feather. Strength of different reducing agents is to be identified.
- Amount of protein obtained under different reducing agents studied.
- Separation of protein using protein precipitating agent.
- Purification and analysis of the protein obtained

1.5 Rationale And Significance

Protein shortage for food force scientist to look for new protein sources, including waste products. Feathers are bio-resource with high protein content (more than 750 g kg-1 crude protein). Keratin is the main component of feathers, representing nearly 90% of feather weight. Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan. The feathers constitute up to 10% of total chicken weight, reaching more than 7.7×108 kg/year as a by-product of the poultry industry. This excessive material is discarded in several cases, being a material of difficult degradation that may become an environmental problem. These hard keratins of chicken feather which are recognized as a solid wastes generated from poultry processing industry are insoluble and resistant to degradation by common proteolytic enzymes, such as trypsin, pepsin and papain because of their high degree of cross-linking by disulfide bonds, hydrogen bonding and hydrophobic interactions. Keratinous wastes are increasingly accumulating in the environment generated from various industries. To recycle of such wastes, biotechnological alternatives are developing to hydrolyze those materials to soluble into natural proteins. Current commercial production of chicken feather protein involves treatment at elevated temperatures and high pressure, this energy intensive process, results in the loss of some essential amino acids. Natural protein obtained from these feather act as true protein thus have a wide range of usages in fields like cosmetics, food, medicine and biotechnology.

CHAPTER 2

LITERATURE REVIEW

2.1 Feathers.

Feathers are among the most complex integumentary appendages found in vertebrates and are formed in tiny follicles in the epidermis, or outer skin layer, that produce keratin proteins. The β-keratins in feathers, beaks and claws — and the claws, scales and shells of reptiles — are composed of protein strands hydrogen-bonded into β-pleated sheets, which are then further twisted and crosslinked by disulfide bridges into structures even tougher than the α-keratins of mammalian hair, horns and hoof. Keratin refers to a family of fibrous structural proteins. Keratin is the key structural material making up the outer layer of human skin. It is also the key structural component of hair and nails. Keratin monomers assemble into bundles to form intermediate filaments, which are tough and insoluble and form strong unmineralized tissues found in reptiles, birds, amphibians, and mammals.

The keratin found in feather is called "hard" keratin. This type of keratin does not dissolve in water and is quite resilient. So what is keratin made from? Keratin is an important, insoluble protein and it is made from eighteen amino acids. The most abundant of these amino acids is cystine which gives hair much of its strength. Keratin filaments are abundant in keratinocytes in the cornified layer of the epidermis; these are cells which have undergone keratinization. Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan.

- The *α-keratins* in the hair (including wool), horns, nails, claws and hooves of mammals.
- The harder *β-keratins* found in nails and in the scales and claws of reptiles, their shells (chelonians, such as tortoise, turtle, terrapin), and in the feathers, beaks, claws of birds and quills of porcupines. (These keratins are formed primarily in beta sheets. However, beta sheets are also found in α-keratins.)

Amino acids present in feathers:

Cysteine	Aspartic acid
Serine	Alanine
Glutamic acid	Proline
Threonine	Isoleucine
Glycine	Tyrosine
Eucine	Phenylalanine
Valine	Histidine
Arginine	Methionine

Table 2.1: Amino acids found in feather Protein.

These feathers are consisting of crude proteins mainly. Researches has been done all over the world to make use these protein content which is a wonderful idea since at the same time bothprotein shortage and waste feathers environmental problems can be overcome.

Constituent	Feathers	FPC
Water	495	55
Crude protein	894	95
Fibers	-	6.8
Fat	14.1	13.1
Ash	62.4	87.7
Ca	3.5	3.4
P	1.3	1.1
Na	4.0	14.2
Cl	8.0	22.2

Table 2.2: Chemical composition of feathers and feather protein concentrate (FPC)

2.2 Protein

Protein is a part of every cell in living organism's body, and no other nutrient plays as many different roles in keeping living things alive and healthy. The importance of protein for the growth and repair of muscles, bones, skin, tendons, ligaments, hair, eyes and other tissues is proven since a very long time. Without it, one would lack the enzymes and hormones needed for metabolism, digestion and other important processes. Natural proteins are proteins purified from natural *sources*.

Highly purified for use in molecular biology and immunology researches. Natural proteins quickly were considered useful ingredients for creating a suitable environment for healthy skin and hair because of their ability to bind water with the horny layer of skin and its annexes. Most protein derivatives that are used for cosmetic purposes are obtained from simple proteins, whereas conjugated proteins are used far less frequently.

Figure 2.1: Normal protein sources.